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ADDITIONAL NOTES ON THE BACTERIOLOGY AND PATHOLOGY OF MILKSICKNESS.*

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INASMUCH as the literature on milksickness has been reviewed by Dr. Jordan and Dr. Harris in the preceding paper, such an introduction to the present paper is not necessary. Early in the autumn of 1908 I began at the suggestion of Dr. Jordan[1] the experimental feeding of dogs with what was believed to be the specific germ of milksickness. Since milksickness is primarily a disease of horses and cattle and is undoubtedly of intestinal origin I almost simultaneously instituted a search for *B. lactimorbi* on the various forage plants which serve as chief nutriment for these animals. This search was in a great measure successful. Various strains of this bacillus isolated from grasses and weeds were compared with the strains isolated by Jordan and Harris from the organs and tissues of animals dead and sick from milksickness, to determine whether possibly certain cultural differences might be revealed which would more or less sharply differentiate "pathogenic" from the widespread "saprophytic" varieties or strains. During this study certain cultural and biochemical features not described in the previous papers were observed. These new features together with the rather limited study of the distribution of the organism in nature and the technic adopted in isolation are embodied in the first half of the present paper. The latter half of the paper presents the results of the experimental work on dogs.

BACTERIOLOGY.

Distribution of the Organism and Manner of Isolation.—The organism was isolated with the greatest ease from two samples of dried alfalfa from Wisconsin ("A. H." and "A. K."); from dried alfalfa

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[1] I wish to thank Dr. E. O. Jordan for helpful suggestions and encouragement during the progress of the work.

from Illinois ("A. M."); from a sample of very old dried alfalfa from Indiana ("A. I."); from *Bigelovia*, *Solanum elaeagnifolium*, *Gutierrezia sphaerocephala*, and *Portulaca pilosa* from the Pecos Valley, New Mexico; and twice from samples of Roquefort cheese. Timothy hay, red clover, bran, hominy, and silage gathered from the same farm from which the alfalfa showed practically nothing except *B. lactimorbi*, were examined bacteriologically for *B. lactimorbi* with negative result. A sample of alfalfa from Oregon where milksickness has never been known to exist showed no organism in any way similar to *B. lactimorbi.*

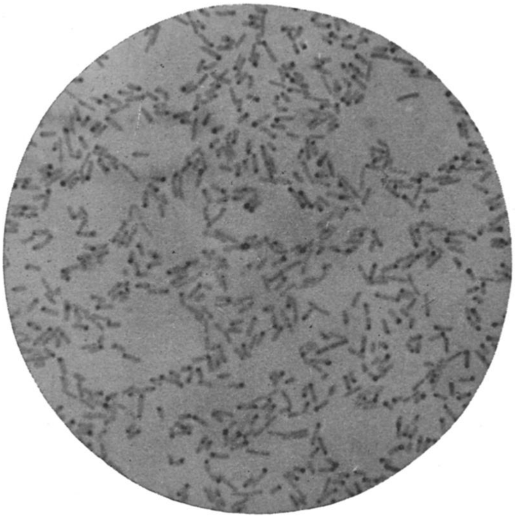

FIG. 1.—*B. lactimorbi* from Indiana alfalfa; 18-hour growth on agar at 30° C. Löffler's methylene blue. ×1100.

The following method was adopted in isolating the organism from plants, e. g., from alfalfa. The more finely divided particles of the dried hay were carefully strewn over the surface of a slant agar tube taken directly from the sterilized bottle in which the samples were collected. The tube inoculated in this manner was then incubated at 30° C. for 18 to 24 hours. At this temperature *B. lactimorbi* if present outgrew the other organisms from the alfalfa so that the dull semi-transparent film-like growth which spread out from the edges of the small particles of the hay consisted almost entirely of a pure culture of *B. lactimorbi.* When a coverslip preparation from this film-like outgrowth was stained with methylene blue, the identity of the organism was easily determined by the presence of the characteristic oval drumstick spores and the presence of metachromatic granules (Fig. 2). The metachromatic granules are particularly large and distinct at the time of and for a short period after isolation (Fig. 3). A loop of this growth was transferred to broth and immediately plated out in plain agar. The growth on the plates showed the organism to be present in almost pure culture. After isolation in pure culture each strain was transferred to the ordinary culture media and the reactions observed compared with the cultural features and biochemical reactions of the organism as described by Jordan and Harris in their first paper.[1] On the whole the reactions were the same. Such differences as were observed together with additional reactions not noted in the previous descriptions will be mentioned here.

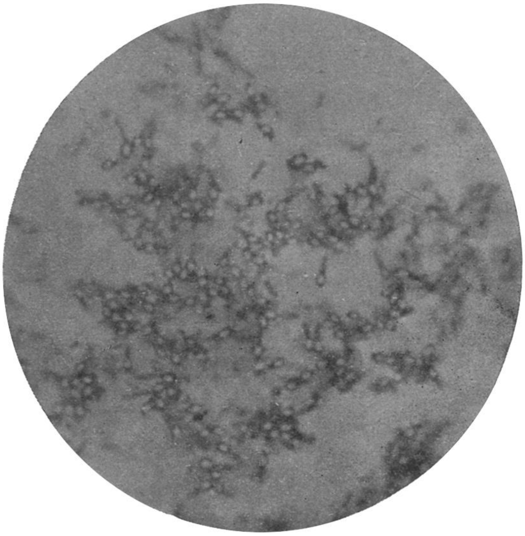

FIG. 2.—*B. lactimorbi* from *Bigelovia rusbyi*, Pecos Valley, N. M.; 24-hour growth on plain agar at 30° C. Löffler's methylene blue, no heat. ×1100.

Agar slant.—Not all strains when grown at 37° or 30° C. show a "delicate veil-like growth" which more or less irregularly covers the surface of the agar. The growth may be entirely confined to the needle track as in the case of the strains isolated from

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[1] *Jour. Amer. Med. Assoc.*, 1908, 50, p. 1665.

Bigelovia and *Gutierrezia sphaerocephala;* or if spreading, the growth may be thick and almost viscid and as regards luster can be easily mistaken for *B. subtilis* (Strain: "Por." and "Sol.").

Litmus milk.—Altho coagulation of the milk is not observed with most strains, the strain isolated from "Portulaca," "Solanum" and the strain "A. I." coagulated milk within 72 hours. The reaction of the milk was strongly alkaline. The strain isolated from *Bigelovia* produced in milk a small amount of a gelatinous sediment. With the increasing growth of the organism and the consequent increase of free alkali production the casein again goes into solution, giving the milk a brownish semi-translucent appearance. If flasks containing 50 c.c. of plain milk are inoculated and incubated for three weeks or longer at 30° C. the milk turns a light pink or dirty brown color. Such cultures give off a putrescent, rancid, and sour odor which in some instances closely resembles the odor of butyric acid. The milk cultures were repeatedly tested for acetone by means of Gunning's iodoform test, for it was thought possible that the rapid emaciation with the occurrence of acetone in the breath and urine during milksickness might be due to the decomposition of the fats of the body into acetone or acetone-like bodies through the agency of *B. lactimorbi.* The tests were, however, negative. The great alkalinity produced in the milk is due to the decomposition of the proteins of the milk with the production of free ammonia.

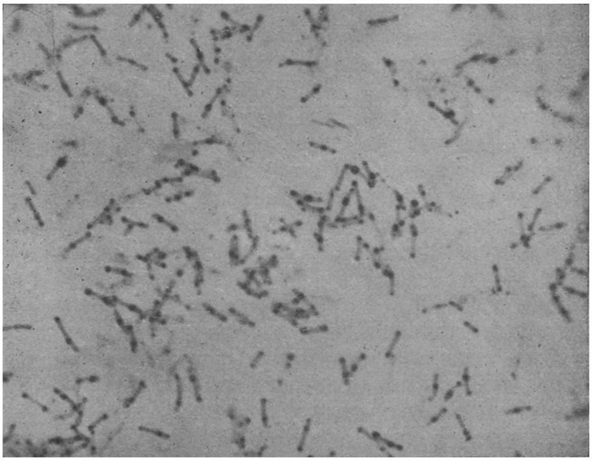

FIG. 3.—*B. lactimorbi* from Meeker alfalfa; 1st generation, 18-hour growth on agar at 30° C. Löffler's methylene blue, no heat. ×1100.

Potato.—In their first communication Jordan and Harris reported the absence of growth of the organism on potato. At that time undoubtedly the organism was of true parisitic nature as regards its food supply and found ordinary potato an unsuitable medium for development. Since then I have had no trouble in obtaining a good and in most instances a luxuriant growth on potato with the original strains and with all strains since then isolated by Jordan and Harris, and myself.[1] In general there appears after 24 hours' incubation at 30° C. on the surface of the potato a greyish-yellow to reddish-brown (depending on the strain) moist growth which spreads slightly from the needle track. Even at this early stage there is noticeable a slate-blue or brownish discoloration of the medium which increases until on the sixth day the entire medium is discolored. Growth is usually accompanied by the production of a very rancid sour odor, tho this is not a significant feature. Smears made from the potato cultures on the sixth day show numerous involution forms with large metachromatic granules similar to the involution forms found after six days' growth of the organism on Löffler's blood serum. Large metachromatic granules, extruded from the cell body, were found massed together in large clusters. At other times minute metachromatic granules were irregularly scattered throughout the greatly elongated and coiled bacterial cell. The granules take an intense blue or reddish stain when treated with methylene blue or with Neisser's stain.

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[1] This may be due to age or variety of potatoes used.

Heinemann's synthetic potato medium.—A good visible growth could be made out at the end of six days. The amount of growth naturally depended on the strain under investigation, being directly comparable to the luxuriance of the growth of the same strain on more favorable culture media. The growth was moist and glossy and had a yellowish to a brownish coloration. The growth was in the majority of the cases strictly confined to the needle track. There was no discoloration of the medium.

Fermentation tubes with sugar broths.—The organism grows equally well in plain, dextrose, lactose, and saccharose broths. At the end of 24 hours' incubation at 30° C. the medium in the bulb is uniformly turbid. Scum formation is pronounced. No growth takes place in the closed arm of the fermentation tube.

Nitrate broth.—Eight strains were grown in nitrate broth to determine whether the organism possessed the power of reducing nitrates to nitrites. The result was negative.

Indol.—Growth of the organism in standard peptone solution failed to reveal the slightest trace of indol production at the end of the tenth day and at the end of three weeks when tested with sulfuric acid and sodium nitrite or with paradimethylamidobenzaldehyde. There was, however, a noticeable amount of ammonia produced when tested with Nessler's reagent as early as 10 hours after incubation at 30° C.

Blood agar plates.—On blood agar plates the colonies have a white center and an irregular periphery. They closely resemble small colonies of *B. subtilis.* A halo-like zone of hemolysis appears immediately about the periphery of the colony. The alkali liberated during the growth of organism is most probably responsible for the hemolysis.

Reaction Inhibitory to the Growth of *B. lactimorbi.*—The reaction inhibitory to the growth of the organism was determined both on solid and in liquid media. Eight strains were examined. On nutrient agar the most luxuriant growth took place when the reaction was alkaline (−3.0, −2.5, −2.0 per cent). With an increase in the acidity of the agar there was scanty growth. The organisms were swollen, of indefinite outline, or in the act of sporing. Growth ceased entirely at +2.0 per cent. Similar results were obtained when the organism was grown in sugar-free broths of various degrees of alkalinity and acidity. Altho there was observed a slight variation as regards the tolerance of the organism for acid or alkali according to the strain examined, it can be laid down that on the whole the organism grows best in an alkaline medium. Some varieties refused to multiply in broth containing more than 10 to 15 c.c. normal HCl acid per liter. Other strains grow well in 15 c.c. Scum or distinct pellicle formation was observed only when the organism was grown in an alkaline broth.

Relation to Free Oxygen.—The feeble growth in the depth of the glucose-agar stab and the absence of growth in the closed arm of the fermentation tubes indicate that the organism is aerobic in nature. Five strains were grown for six days at 30° C. in a Novy jar containing H_2. No growth was observed at the end of that period. The organism can, therefore, be designated as an obligatory aerobe.

EXPERIMENTAL FEEDING OF DOGS.

Dogs were used exclusively in the feeding experiments to be reported. Prior to the feeding with sporogenous gelatin cultures of *B. lactimorbi* all animals were put under careful observation eleven days or more. During this period loss or gain in weight, disposition, appetite, and character of feces were noted, and served as a check on symptoms which followed the feeding of the organism.

Dog 5.—Fox-bull-terrier. Male, well nourished, healthy. Weight on the 1st, 5th, and 11th days, respectively: 10 kgs. 260 gm., 10 kgs. 200 gm., 10 kgs. 200 gm. Gentle, timid, excellent appetite, excretions normal. Protocol follows:

November 22, 1908: Received 80 c.c. of a four-day sporogenous gelatin culture of *B. lactimorbi* ("Alva"). General condition good. Weight, 10 kgs. 200 gms. Excretions soft. Disposition normal. Appetite good.

November 23, 24, 25: Dog received 50 to 80 c.c. of sporogenous culture of *B. lactimorbi.* No change from the normal.

November 26: Lethargic. Trembles. Hind legs particularly involved in the tremors. Looks rather emaciated. Retches repeatedly. Vomitus consists of stringy "cotton" mucus. In a drowsy condition. Refuses to leave the box. Not vicious. Attentive to call. Fifty c.c. of a sporogenous gelatin culture of *B. lactimorbi* ("Alva") were mixt with the food and placed in the box. Appetite fair. Retching ceased. Feces small in amount and semi-solid.

November 27: General condition about the same as on the preceding day. Both eyes inflamed. Left sclerotic deeply injected. Disposition unchanged. Scant feces are semi-solid in nature. Refuses to eat or drink.

November 28: General condition unchanged. Easily fatigued. Walked slowly up two flights of stairs, but refused to walk the third flight. Very thin and emaciated. Mucus discharge from the eyes and nose. Coughs and retches after slightest exertion. Attentive. Pricks up ears at the call of whistle. Prefers to be left alone. Refuses to eat. Drinks freely of water. Constipated. After great effort passed 5–10 gm. of a semi-solid brown colored feces which contained *B. lactimorbi* in great numbers. Weighs 7 kgs. 820 gm., i. e., lost 2 kgs. 380 gm. in six days.

November 29: The animal was found dead at ten o'clock A. M., and in my absence was examined by Dr. Jordan and Dr. Harris to whom I am indebted for the following report of the gross, microscopic, and bacteriological findings.

"Animal not entirely cold. Rigor mortis present. Extremely emaciated. External evidences of having passed thin brownish feces. No subcutaneous fat. Muscles of thorax deep red. No odor of acetone.

Abdominal cavity.—No peritonitis, no excess of fluid, no visceral or parietal ecchymoses. Omentum almost devoid of fat. Intestines throughout empty of feces, caliber small. *Small intestine* excised and opened through the entire length. There was much sticky yellow (bile-stained) mucus everywhere but particularly in the duodenum and upper one-third of the gut; also numerous flat and round worms. On washing, the duodenal portion was much injected, and the mucosa edematous; there were no definite hemorrhages in the mucous membrane, and no erosions. There was no definite folliculitis in the tract. Injected areas were found here and there widely separated. Peyer's patches were not involved. They lay below the general level of the mucosa, and were of a brownish-gray color. *Large intestine and appendix:* Altho outwardly not showing any evidences of an abnormal state, the whole tract appeared somewhat edematous, but more striking was the degree and extent of the injection of the mucosa, which in one or two places actually constituted a hemorrhage. No folliculitis could be demonstrated. The appendix presented a similar injected condition. The colon was preserved in Kaiserling's solution. The *mesenteric glands* were moderately enlarged. The *liver* could not be said to be enlarged. Its color was a deep maroon. It was friable. On section it dripped much blood and altho the lobules could be seen there was some degree of cloudy swelling. *Spleen* not enlarged, pale, and of normal consistence. Section disclosed no abnormalities. *Kidneys:* Both looked alike; enlarged and congested, of a deep maroon color. On section much blood oozed out and there was a considerable degree of cloudy swelling. Interlobular vessels distinct, Malpighian tufts seen with difficulty. *Adrenal glands* pale in color.

Thorax.—The lungs showed a small degree of collapse on opening of the chest. They presented a mottled appearance, deep red areas amid lighter ones; and in these deep red areas grayish areas about 2 mm. could be seen scattered about; these latter were soft and on section appeared purulent. Evidently a condition of bronchopneumonia. There were signs of an early pleurisy in the left cavity of the thorax. On section of both lungs numerous areas of a deep red color were seen, as well as mucopurulent contents in the smaller bronchi. *Heart and pericardium:* No excess of fluid in the pericardial sac. No pericarditis. No ecchymoses. Right side of the heart was distended, left collapsed. One or two pin-point ecchymoses at the roots of the great vessels. Color of the organ darker than normal. On section the muscle looked a trifle darker than normal and showed a mild degree of cloudy swelling. Its consistence was softer than normal. The anterior cusp of the tricuspid valve gave evidence of an old healed endocarditis. On one of the cusps (ventral side) of the pulmonary artery was a subendocardial hemorrhage of an irregular size, linear in extent,about ½ by 3 mm.

Tissue samples were preserved for study in 10 per cent formalin and in 95 per cent alcohol. The paraffin sections of the tissues were stained with hematoxylin and eosin.

MICROSCOPIC. *Liver.*—Hyperemic. Otherwise normal.

Colon.—Normal.

Small intestine.—Normal.

Heart.—Some bundles of fibers appear to be normal. Others are fibrillated and contain many small fat droplets.

Kidney.—A moderate grade of acute glomerulonephritis, the parenchymatous changes being chiefly in the convoluted tubules, altho others of the loops of Henle and collecting tubules are affected. In places there is complete necrosis of the epithelium.

Spleen.—Greatly hyperemic, even the Malpighian bodies are to a considerable extent infiltrated with blood. Some brown pigment scattered about the section within the cells for the most part.

Cerebrum.—Appears normal.

Lung.—Section taken through a superficial purulent area is filled with round and polynuclear leukocytes. Aveolar capillaries hyperemic. The pleura is only slightly nflamed.

BACTERIOLOGICAL.—Cultures were made on agar from the heart, bronchopneumonic areas in the left lung, left pleura, liver, bile, kidney, mucus of the small intestine. *B. lactimorbi* was isolated from liver, spleen, and intestinal mucus."

Dog 6.—Black and white. Bitch, alert, slender build. Weight constant during period of "control" observation. Affectionate, appetite normal, feces normal. From November 11 to December 27, 1908, received daily an average of 135 c.c. of a four-day sporogenous gelatin culture of *B. lactimorbi* ("Ph^1-Bile"). Dog never showed the least sign of being ill. Early in January the animal was etherized and immediately examined. Since the organs revealed no gross anatomical lesions they were not examined microscopically. The serum possessed a high agglutinating power for the "Alva" strain as can be seen from Table 1.

TABLE 1.

STRAIN	SERUM	AGGLUTINATION OBSERVED AFTER 24 HOURS						
		1:10	1:50	1:100	1:500	1:1,000	1:5,000	Control
"Alva"	Normal Dog	+	–	–	–	–	–	–
"Alva"	Dog 6	+	+	+	+	+	–	–
"Alva"	Normal Human	–	–	–	–	–	–	–

Unfortunately a 0.9 per cent NaCl suspension of the "Bile" strain with which the dog had been fed agglutinated spontaneously and it was necessary to test the agglutinating action on various other strains of *B. lactimorbi.* The following table gives the result of the experiment:

TABLE 2.

SERUM OF DOG 6	AGGLUTINATION AFTER 24 HOURS						
	1:10	1:50	1:100	1:500	1:1,000	1:5,000	Control
Strain "Alva"	+	+	+	+	+	–	–
Strain "Ontario"	+	+	–	–	–	–	–
Strain "H. J."	+	+	–	–	–	–	–

Normal dog's serum possessed no agglutinating power on *B. lactimorbi* in a greater dilution than 1:10; normal human serum possessed no agglutinating action whatever. We have here, possibly, an instance of an organism not sufficiently pathogenic to cause disease but able to stimulate the production of specific agglutinins.

Dog 7.—Brown, sleek, well-nourished bitch. Weight: 9 kgs. 800 gm. Constant during the period preliminary to the feeding of *B. lactimorbi.* Excellent appetite, affectionate. From November 11 to December 28, 1908, received daily 180 c.c. of a four-day sporogenous culture of *B. lactimorbi* (Strain: "Brain"). Shortened protocol follows:

November 12–December 13, 1908: Nothing abnormal. Increase in weight due to pregnancy.

December 14: Gave birth to three pups. Remained in the box with young. Shivers occasionally. Vicious when persons come near box. Refuses to eat. Feces well formed.

December 15: Back to normal condition. Remained so until December 26.

December 26: Refuses to leave box. Face looks drawn. Slight tremors of the hind limbs. Wags tail and pricks up ears at call of whistle. Coaxed out of box once. On the whole in a drowsy, stuporous condition. Prefers to be left alone. Closes eyes while in a standing position as if going to sleep. Vicious when other dogs come near the box. Attentive to the young. Feces partly solid, partly soft. Feces contain little mucus.

December 27: Feces very soft. Animal in normal condition.

December 28: Feces semi-solid and blood stained. General condition normal.

From December 28, 1908, to February 2, 1909, dog remained in good health. The dog was killed with ether on February 3 (six weeks after giving birth to the pups and about one month after the last feeding with gelatin cultures of *B. lactimorbi*).

AUTOPSY.—Well-nourished bitch. Weighs 10 kilos. Has bad case of mange. Complete physiological atrophy of the mammary glands. No external abnormalities.

Abdominal, pleural, and pericardial cavities normal. *Heart and lungs* normal. Lungs are slightly congested. *Peribronchial lymph glands* are not enlarged, slightly anthracotic. *Mesenteric and retroperitoneal lymph glands* are not enlarged.

Gastro-intestinal tract, spleen, and kidneys normal.

Liver.—About normal in size. Mottled, yellow and white areas alternating with reddish areas. The liver is exceedingly yellow. There are several patches of irregular outline (2 by 3 cm. and smaller) which are distinctly white. The liver has a fatty appearance and touch. The lobules are well marked. Liver parenchyma exceedingly friable. Cut surface of the liver has a yellowish appearance. Small congested areas noted on the external surface are found to be only subserous. Gall-bladder is full of a thick golden-colored bile. No concretions in the gall-bladder.

Urinary and generative tract normal.

ANATOMICAL DIAGNOSIS.—Mange. Slight fatty infiltration of the liver.

MICROSCOPIC.—Celloidin sections. Hematoxylin and eosin.

Myocardium normal.

Lungs.—Alveolar capillaries highly congested and contain numerous polymorphonuclear leukocytes. Otherwise normal.

Liver.—Naked-eye examination of the stained sections shows a strikingly mottled appearance. On microscopic examination it is found that the mottled appearance is due to the fact that the central portions of the lobule take on a fainter hemotoxylin and eosin stain than does the periphery of the lobule. The cells near the central vein are slightly infiltrated with fat. Sections of the tissue taken from the periphery of the organ show some cells having the typical signet-ring appearance. Here there is also subcapsular congestion. In some places the protoplasm of the cells is entirely replaced by fat globules. There is no congestion of the intralobular vessels. Marked leukocytosis. Scattered throughout the section of the liver are small foci or aggregates of round cells. There are approximately 20 to 30 cells of the lymphoid type to such a focus. The nuclei of these round cells stain intensely with hematoxylin. In some of the foci can be seen epitheloid cells and polymorphonuclear leukocytes. These foci are not limited to any particular part of the section, appearing at one time near the central vein, at another time at the periphery of the lobule. The interlobular connective tissue is normal in amount. The numerous foci of round cells together with the pronounced leukocytosis suggested the possibility of *B. lactimorbi* within the foci. Sections of the liver were stained for bacteria. No organisms were found within the foci.

Spleen normal.

Kidneys.—Blood vessels of the kidneys are highly congested. Glomeruli are somewhat contracted. Renal epithelium normal. There are no casts.

BACTERIOLOGY.—Cultures made from the liver, spleen, and kidney were found sterile. *B. lactimorbi* could not be isolated from the intestinal mucus.

Pups.—As recorded in the preceding protocol, Dog 7 gave birth to three pups. One of the pups was found dead the morning after the delivery. An autopsy was immediately made.

Extensive hemorrhage into the anterior abdominal wall about the umbilicus. The hemorrhagic area was 3 cm. long and 2 cm. wide. Condition of general anasarca with hydrothorax and great amount of clear watery ascitic fluid. The pup was still-born as shown by the floating test. The organs were slightly pink in color and flabby. Beyond this no anatomical lesions were found. Cultures made from the liver were sterile. *B. lactimorbi was, however, isolated from the peritoneal transudate and from the kidney.* Unfortunately no tissues were kept for microscopic examination.

The remaining two pups were in a poor condition, in part due, no doubt, to the fact that they were poorly nourished by the mother. After the mother had been autopsied they were immediately put on a rich diet to increase their general condition. Unfortunately one of the pups died from injuries received by falling from its cage. The condition of the other pup gradually improved and feeding was begun on February 22 with milk and gelatin cultures of the "Alva" strain. The pup received daily about 50 c.c. of a five-day milk or gelatin culture of *B. lactimorbi.* Feeding continued uninterruptedly until May 1. The pup never showed any signs of being sick. On March 25 some blood was withdrawn and its agglutinating power determined for various strains of *B. lactimorbi*, with the following result:

After 34 hrs. agglutination complete in 1:500 for "Alva" strain.
" " " " in 1:200 for "Brain" strain.
" " " " in 1:50 for "Ontario" strain.
" " No agglutination in 1:10 for "H. Park" strain.
" " " in 1:10 for "A. W." strain.

AUTOPSY (May 22). **Dog 8.**—*Age:* 5 months. *Weight:* 4,950 gm. Growth markedly stunted. Subcutaneous fat abundant. Peritoneal, pleural, and pericardial cavities contain the normal amount of clear fluid. The internal organs with the exception of the liver showed no pathological changes. The liver was soft and friable. Had a slight pink color. Aside from a dull yellowish pink coloration cut surface showed no gross anatomical lesions. Outline of lobules not to be made out. In consistence the liver was soft. Large patches of the capsule of the liver were easily torn from the surface of the liver.

Organs were sterile. *B. lactimorbi* could not be isolated from the gastrointestinal tract. Pieces of the various organs and tissues were fixed in Zenker's, imbedded in celloidin, and the section stained with hematoxylin and eosin.

MICROSCOPIC.—*Kidneys, lungs and myocardium* normal.

Liver.—Contains very little blood. There is no sharp line of separation between individual lobules. Normal lobular structure with the trabeculae of liver cells alternating with the intralobular capillaries is absent. The lobule seems to be made up entirely of large highly granular fatty liver cells with rather indistinct nucleis. The swollen liver cells have led to an almost entire obliteration of the intralobular capillaries. Connective tissue normal in amount.

Dogs 9 and 10.—These dogs were fed daily with large quantities of *B. lactimorbi.* On an average each dog received 180 c.c. of a four-day sporogenous gelatin culture of the organism. Dog 9 received cultures of the "H. J." strain isolated by Jordan and Harris from a human being dead from milksickness; Dog 10 received gelatin cultures of the organism isolated from the spleen of Dog 5. In spite of these large quantities of the organism fed daily to the dogs for a period of three weeks, neither dog ever showed the slightest degree of illness. Experimentation was therefore discontinued and the dogs were autopsied. No gross anatomical lesions were found.

DISCUSSION AND SUMMARY.

It is remarkable that *B. lactimorbi* with its pronounced and characteristic morphological and tinctorial features and its wide distribution in nature should have escaped notice for so long a time. C. Günther[1] describes a bacillus isolated by him from water which possessed motility, liquefied gelatin, and formed drumstick spores. A microphotograph of the sporing organism closely resembles *B. lactimorbi.* I have been unable to get the original article in which Günther described the organism in greater detail. Another organism isolated by Grether[2] from canal water, and to which Günther refers as a closely allied form, has cultural features which very closely resemble those of *B. lactimorbi.* This organism repeatedly isolated by Grether from canal water is about 1:5 µ long, grows well at room temperature and at 28° C. but not at 37° C., possesses drumstick spores which in stained preparation appear oval, forms yellow moist growth on potato accompanied by a discoloration of the potato, grows only in the bulb of the fermentation tube containing slightly alkaline dextrose broth, but is not motile, nor does it cause liquefaction of gelatin. The similarity of the organisms described by Günther and Grether to *B. lactimorbi* is apparent and the morphology and the cultural features as far as they are given strongly suggest that they may be related organisms. Owing to its polymorphic tendency the growth of *B. lactimorbi* on agar plates and on agar slants may and often does resemble the luxuriant, spreading growth of *B. subtilis*, and on this account has undoubtedly heretofore been mistaken for *B. subtilis* or for organisms of the subtilis group.

The close association of *B. lactimorbi* with only certain plants as

[1] *Einführung in das Studium der Bacteriologie*, 1898, p. 575; and plate IV, Fig. 23.

[2] *Archiv f. Hyg.*, 1896, 27, p. 226.

noted above is of general bacteriological interest, and certainly invites further investigation. The contrast between plates poured from growth of timothy hay and alfalfa is most striking; the latter contain numerous colonies of *B. lactimorbi*, the former contain practically nothing but the large spreading colonies of *B. subtilis*. Of the five weeds gathered in the Pecos Valley, New Mexico, where the disease was first studied by Jordan and Harris, four (*Portulaca pilosa*, *Solanum elaeagnifolium*, *Gutierrezia sphaerocephala*, and *Bigelovia Rusbyi*) yielded strains of *B. lactimorbi*. *Senecio filifolius* collected from the same region contained no organism in any respect similar to *B. lactimorbi*. The search for the organism on clover, hominy, bran, and silage likewise yielded negative results. From dried alfalfa the organism was isolated with great ease. The samples of alfalfa were collected for the most part from areas where milksickness has never been known to occur and from farms which supply Chicago and Milwaukee with certified milk.

Some of the strains show minor cultural differences. "Por.," "A. I.," and "Sol." caused coagulation of milk after 72 hours. The reaction of the milk is alkaline. Resolution of the precipitated casein was complete on the eighth day, the milk having at the end of this period a dirty brownish turbid appearance. Growth of strains, "Gutierrezia," "Brain," and "Bigelovia," on plain agar slants resemble very much growth of *Strept. pyogenes* on the same medium. Aside from these differences the cultural and biochemical features of the saprophytic strains and of the "pathogenic" strains isolated from man and animal dead or dying from milksickness were identical.

Of the six dogs inoculated two (Dog 5 and Dog 7) showed in a slight degree the symptoms observed in milksickness: stupor, coma, muscular weakness, retching, and loss of appetite. It will be recalled that Dog 5 died as a result of illness. At the autopsy there was found bronchopneumonic condition of the lungs. This constituted the most conspicuous macroscopic and microscopic lesion. Cloudy swelling of the liver and edema of the colon with marked injection of the mucosa which in one or two places actually constituted hemorrhage were the other gross anatomical findings. Microscopically the liver is described as "hyperemic, otherwise normal," and the colon and remaining tissues with exception of lungs are described as being "normal." It is highly probable that the cause of death in this instance was the bronchopneumonia. If we take these points into consideration it is difficult to understand how *B. lactimorbi* isolated from a normal liver, normal spleen, and from normal intestinal tract of this animal should have been the cause of the disease and the primary cause of death when the organism was not present in the distinct bronchopneumonic patches. It is possible that *B. lactimorbi* caused the bronchopneumonic condition and then disappeared from the lesions as there were no bacteria in the areas.

The results of experimentation with Dog 7 are more decisive. This dog was taken sick with symptoms slightly simulating the symptoms observed in milksickness, but recovered. At the autopsy the animal showed a highly friable liver with slight fatty infiltration. Microscopic examination of the liver revealed (aside from slight fatty infiltration) numerous healed focal necroses. Moreover, this dog gave birth to three pups one of which was stillborn. *B. lactimorbi* was isolated from the peritoneal transudate and from the kidney of this pup.

Dog 8 (pup) on the other hand never showed the slightest degree of illness in spite of the fact that the dog received large quantities of the organism daily for a very long period of time. Altho no illness resulted there was a pronounced retardation of growth. The serum of the dog, likewise, possessed a rather high agglutinating power for several strains of *B. lactimorbi.* At autopsy the liver was found very soft and friable. In all other respects the dog was normal. Histologically the tissues were normal with exception of the liver. The liver showed parenchymatous degeneration and beginning fatty degeneration.

Dogs 6, 9, and 10 never exhibited the slightest degree of illness. Organs and tissues were found normal. It is true that the agglutinating power of the serum of Dog 6 lay between 1:1,000 and 1:5,000 for the "Alva" strain. It is, however, entirely probable that the body would react even against the most saprophytic organism when given in large quantities with the production of specific agglutinins which would cause sedimentation even in high dilution. The same may also explain the high agglutinating power of the serum of Dog 8 (pup).

The preceding experiments are, I believe, far from being decisive in establishing *B. lactimorbi* as the etiological factor in the production of milksickness. The organism either loses its pathogenicity very rapidly when grown on artificial media; or the virulence of the organism is dependent on what may be termed a symbiotic life or existence on certain plants. It is possible that its growth on such plants as the rayless goldenrod (*Bigelovia*), white snake root (*Eupatorium ageratoides*) is responsible for its pathogenic power. Both of these plants have been thought to be the cause of milksickness, and bacteriological examination of the rayless goldenrod from the Pecos Valley, New Mexico, demonstrated that at least half of the organisms found associated with this plant consisted of *B. lactimorbi.* I have had no opportunity of examining bacteriologically *Eupatorium ageratoides.* This plant grows abundantly about Joliet, Ill., where milksickness is more or less prevalent throughout the year. Whether the bacillus when growing on certain plants experiences an increase in its pathogenic power or whether it produces from the plant substance a toxic material which is responsible for the symptoms of the disease can only be offered as conjectures. On the other hand, it is remarkable that if *B. lactimorbi* be the cause of milksickness, it should have so wide a distribution in milksick and non-milksick regions. It has also been apparent that loss of virulence occurs after a time in the races of the organism isolated, and that no feeding experiments with several strains of *B. lactimorbi* have so far yielded uniformly any clear-cut pathological picture of the disease. Up to the present no method has been devised which will increase the virulence of the organism.